Purification and Partial Characterization of Lacticin 481, a Lanthionine-Containing Bacteriocin Produced by *Lactococcus lactis* subsp. *lactis* CNRZ 481

J.-C. PIARD,[1]* P. M. MURIANA,[2]† M. J. DESMAZEAUD,[1] AND T. R. KLAENHAMMER[2]

Station de Recherches Laitières, INRA, 78352 Jouy-en-Josas Cedex, France,[1] and Department of Food Science, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, North Carolina 27695-7624[2]

Lacticin 481, a bacteriocin produced during the growth of *Lactococcus lactis* subsp. *lactis* CNRZ 481, was purified sequentially by ammonium sulfate precipitation, gel filtration, and preparative and analytical reversed-phase high-pressure liquid chromatography. Ammonium sulfate precipitations resulted in a 455-fold increase in total lacticin 481 activity. The entire purification protocol led to a 107,506-fold increase in the specific activity of lacticin 481. On the basis of its electrophoretic pattern in sodium dodecyl sulfate-polyacrylamide gels, lacticin 481 appeared as a single peptide band of 1.7 kDa. However, dimers of 3.4 kDa also exhibiting lacticin activity were detected. Derivatives of the lacticin-producing strain which did not produce lacticin 481 (Bac⁻) were sensitive to this bacteriocin (Bacˢ) and failed to produce the 1.7-kDa band. Amino acid composition analysis of purified lacticin 481 revealed the presence of lanthionine residues, suggesting that lacticin 481 is a member of the lantibiotic family of antimicrobial peptides. Seven residues (K G G S G V I) were sequenced from the N-terminal portion of lacticin 481, and these did not shown any homology with nisin or other known bacteriocin sequences.

A major trait of lactic acid bacteria justifying their extensive use in dairy, meat, and vegetable fermentations is their inhibitory activity towards a variety of food spoilage microorganisms. Among the different types of inhibitors produced by lactic acid bacteria (21, 27, 28), bacteriocins have gained increasing interest. These substances exert a broad spectra of inhibitory activity among bacteria, and their proteinaceous nature implies their degradation in the gastrointestinal tracts of man and animals. Thus, antimicrobial proteins produced by starter cultures are excellent candidates to improve the safety of various fermented foods. Numerous bacteriocins are produced by lactic acid bacteria (18, 28), and within the genus *Lactococcus*, nisin has been the most extensively studied bacteriocin. It is active against gram-positive bacteria and can appear as several types (nisin A, B, C, D, or E) that differ in both amino acid composition and biological activity (12). Nisin A is a 34-amino-acid peptide containing lanthionine and β-methyllanthionine residues (9). Pronisin is synthesized ribosomally and undergoes posttranslational modification such that serine and threonine are dehydrated to dehydroamino forms, which then condense with cysteine to give lanthionine or β-methyllanthionine, respectively (13, 15). Other lactococcal strains produce bacteriocins with a more restricted spectra of inhibitory activity and different physical and chemical properties (6, 8, 19). Recent studies in our laboratory have characterized lacticin 481, a bacteriocin produced by *Lactococcus lactis* subsp. *lactis* CNRZ 481. This substance exerted a bactericidal activity against *Lactococcus* species, some lactobacilli, leuconostocs, and *Clostridium tyrobutyricum* (26). Cultures of *L. lactis* subsp. *lactis* CNRZ 481 maintained at a constant pH of 5.5 exhibit maximum production of lacticin 481. When

the producer strain was combined in a cheese starter with a lacticin-sensitive *Lactococcus* strain, the bacteriocin producer rapidly dominated. One of our objectives is the study of mechanisms that determine the host specificity of bacteriocins. A better understanding of these features at the molecular level requires knowledge of the structure of bacteriocins. This study describes steps for the purification of lacticin 481 and presents our initial characterization of its amino acid composition and sequence.

MATERIALS AND METHODS

Organisms, media, and growth conditions. The bacterial strains used were *L. lactis* subsp. *lactis* CNRZ 481 Bac⁺ Bacʳ (bacteriocin producing and resistant to lacticin 481), described previously (26); *L. lactis* subsp. *cremoris* CNRZ 117 Bac⁻ Bacˢ, a sensitive indicator for assay of lacticin 481 activity; *L. lactis* subsp. *lactis* T₁₁ Bac⁺ Bacʳ, a transconjugant obtained from conjugal matings between *L. lactis* subsp. *lactis* CNRZ 481 and *L. lactis* subsp. *lactis* IL1441 Bac⁻ Bacˢ; *L. lactis* subsp. *lactis* T₁₁₄, a Bac⁻ Bacˢ derivative of *L. lactis* subsp. *lactis* T₁₁ and *Lactobacillus delbrueckii* subsp. *bulgaricus* 1489 Bac⁻ Bacˢ (14). Cultures were maintained as frozen stocks held at −20°C in Elliker broth (Biokar, Pantin, France) supplemented with 15% glycerol (Prolabo, Vitry, France). The strains were propagated twice at either 30°C (lactococci) or 37°C (lactobacilli) before experimental use, and each at a 1% level was inoculated into Elliker broth (lactococci) containing sodium β-glycerophosphate (15 g/liter, EGP medium) or into MRS broth (Biokar) (lactobacilli). Agar medium was prepared by the addition of 1.5% (0.75% for the soft overlay agar) granulated agar (Biokar) to the broth basal medium.

Bacteriocin assay. The most sensitive indicator, *L. lactis* subsp. *cremoris* CNRZ 117, was chosen to assay for lacticin 481 activity. Titrations were performed by the serial twofold

* Corresponding author.
† Present address: Food Science Department, Purdue University, West Lafayette, IN 47907.

TABLE 1. Purification of lacticin 481

Sample	Total biological activity (10^{-2} AU)	Total protein (mg)	Sp act (AU/mg)	Recovery (fold)	Purification (fold) based on:	
					Activity	Protein recovery
Culture supernatant	5,760	1,134	508	1	1	1
60% $(NH_4)_2SO_4$ precipitate	10,240	18	56,888	1.8	112	62
80% $(NH_4)_2SO_4$ precipitate	2,621,440	11	23,831,272	455	46,912	103
Gel-filtrated extract	8,448	1.8	512,000	1.5	1,008	672
C_{18} reversed-phase HPLC extract	655,360	0.12	54,613,333	11.4	107,506	9,430

dilution assay described previously, and activity was assigned as arbitrary units per milliliter (26).

Bacteriocin production. The lacticin 481 producer *L. lactis* subsp. *lactis* T_H was grown in E'GP medium (EGP without gelatin) at 30°C for 9 h at a constant pH of 5.5 with a neutralizer of 3 M NH_4OH. Culture supernatant was collected in the early stationary phase of growth, adjusted to pH 6.0, filtered through 0.22-μm-pore-size filters, and then treated for 10 min at 100°C. The heat treatment provided stability to the lacticin 481 preparations during storage (26). Active supernatant was stored at −20°C until used.

Ammonium sulfate precipitations. Aliquots (200 ml) of culture supernatants held at 4°C were brought to 60% saturation by the addition of solid ammonium sulfate with stirring. The samples were stirred vigorously at 4°C over 4 h and then centrifuged at 20,000 × *g* for 45 min at 4°C. The pellets were resuspended in 20 ml of Sørensen buffer 25 mM (pH 6.0 [32]) and treated with ammonium sulfate to 80% saturation. Following centrifugation (20,000 × *g*, 45 min), the pellet was resuspended in 5 ml of Sørensen buffer and assayed for both bacteriocin activity and protein content (bicinchoninic acid reagent; Pierce, Mallet SA, Roissy, France) according to the manufacturer's specification. To remove salt from the precipitate, the pellet in buffer was loaded on C_{18} Sep-Pak cartridges (Millipore SA, St. Quentin-en-Yvelines, France) and washed with 0.1% trifluoroacetic acid (TFA) (Pierce), and bacteriocin activity was eluted with 2-propanol (80%; Rathburn, Walkerburn, Scotland) in 0.1% TFA.

Gel filtration chromatography. Low-pressure gel filtration was performed with a K16 column (Pharmacia, Les Ulis, France) packed with Ultrogel Aca 202 gel (IBF, Villeneuve-la-Garenne, France). Gel height was 35 cm. The running buffer was Sørensen (25 mM, pH 6.0) containing 150 mM NaCl plus 0.02% sodium azide. Flow rate was adjusted to 9 ml/h, and 2.5 ml of sample was injected onto the column. Three fractions were collected each hour.

Reversed-phase chromatography. The pooled active fractions obtained from gel filtration chromatography were loaded on C_{18} Sep-Pak cartridges and washed with 25% 2-propanol in 0.1% TFA. Bacteriocin activity was eluted with 50% 2-propanol in 0.1% TFA. This extract was concentrated by evaporation in a vacuum centrifuge (Savant Instruments, Farmingdale, N.Y.). Further purification was performed with a reversed-phase high-performance liquid chromatography (HPLC) system (LKB-Pharmacia) utilizing a 30-cm μBondapak C_{18} column (Millipore). For the mobile phase, solvent A was 5% 2-propanol in 0.1% TFA, and solvent B was 95% 2-propanol in 0.1% TFA. A linear gradient which went from 75% A–25% B to 50% A–50% B within 60 min was used. To check the purity of lacticin 481, the peak eluting with bacteriocin activity was analyzed with

the same HPLC system using solvent A at 10% 2-propanol in 25 mM ammonium acetate and solvent B at 60% 2-propanol in 20 mM ammonium acetate (pH 6.0). The gradient consisted of a 40-min linear gradient from 70% solvent A–30% solvent B to 40% solvent A–60% solvent B.

PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (20) in 20% T–0.5% C_{bis} gels. Where indicated, the gels were blotted onto polyvinylidene difluoride membranes (Millipore SA) with a Hoeffer Model TE-52 apparatus, as described by Pluskal et al. (29). Gels or membranes were stained with either Coomassie blue R-250 (Serva, Tebu SA, Le Perray-en-Yvelines, France) or silver stain (Bio-Rad SA, Paris, France). Recovery of purified material from the membrane blots was achieved by cutting the appropriate section of membrane and soaking it for 10 min in 1 ml of methanol-acetic acid-water (90:5:5, vol/vol/vol).

Amino acid composition and sequence analysis. Amino acid analysis of purified lacticin 481 (500 pM) was performed with an amino acid analyzer model 420A (Applied Biosystems, Roissy CDG, France) equipped with an integrated hydrolysis system (6 N HCl under argon atmosphere at 155 to 160°C for 75 min). Cysteine-cystine residues were identified after reduction and alkylation of these residues by using 4-vinylpyridine (10). The pyridylethyl-cysteine derivative eluted just prior to Ile in amino acid analyses. For tryptophan quantitation, the procedure described in (22) was used, and amino acid analysis was performed with an amino acid analyzer model LC 3000 (Biotronik, Maintal, Germany).

Sequencing of lacticin 481 was performed by Edman degradation (7) with an automatic sequencer model 120A (Applied Biosystems).

RESULTS

Partial purification of lacticin 481. The purification steps and recoveries of lacticin 481 are given in Table 1. At 60% ammonium sulfate saturation, a 1.8-fold increase in total lacticin 481 activity was recovered in the pellet, while 98.5% of the contaminating proteins were eliminated. Upon subsequent treatment with ammonium sulfate at 80% saturation, lacticin 481 activity in the resulting pellet measured 455-fold higher than that in the initial active supernatant. Desalting of the active extract with Sep-Pak cartridges provided the identical bacteriocin titer. Thus, a significant increase in specific activity occurred during this step of purification. Taking into account the increase in total activity, the specific activity rose by a factor of 46.912. However, on the basis of proteins levels, it appeared that lacticin 481 had been purified 103-fold.

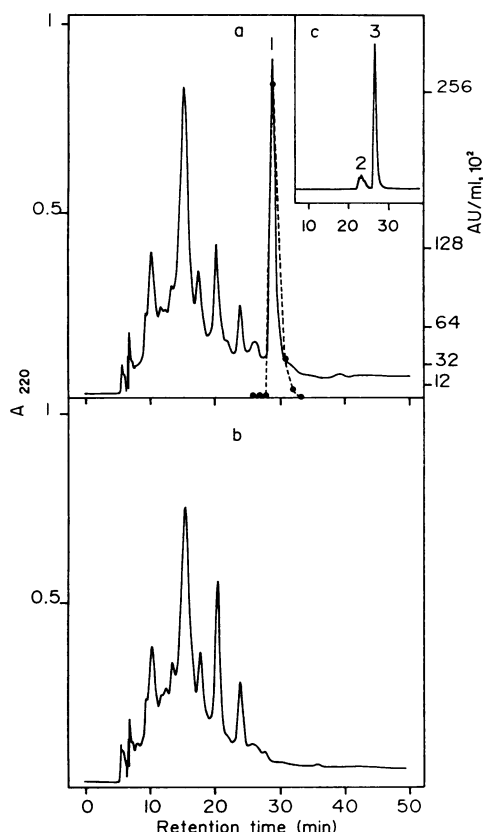**Further purification of lacticin 481.** Gel filtration chroma-

FIG. 1. C_{18} reversed-phase chromatograms of partially purified lacticin 481 from *L. lactis* subsp. *lactis* T_H (a) and an extract from the lacticin-deficient strain from *L. lactis* subsp. *lactis* T_{H4} (b) with a gradient of 2-propanol in TFA. A second purification of peak 1 from panel a with a gradient of 2-propanol in ammonium acetate (c [inset]) is shown. AU, arbitrary units.
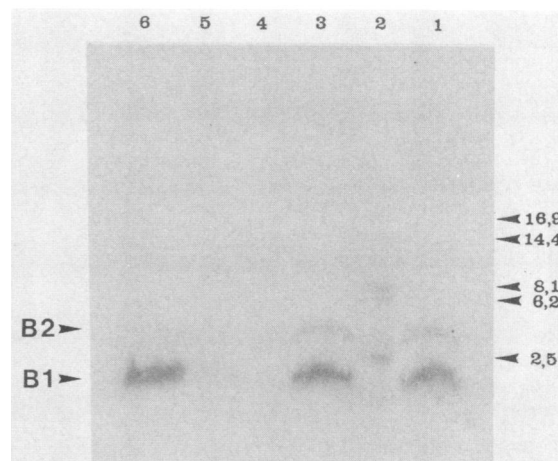

FIG. 2. Coomassie blue-stained membrane containing protein blotted from a SDS-PAGE gel. Lanes: 1 and 3. native and heat-treated aliquots. respectively. of the Fig. 1a, peak 1 fraction; 4, the HPLC extract from *L. lactis* subsp. *lactis* T_{H4} Bac⁻ Bac'; 5, peak 2 from Fig. 1c; 6, peak 3 fractions from Fig. 1c; 2, molecular weight standards whose sizes are given on the right. Two protein bands are visible on the membrane: B1 is a 1.7-kDa peptide and B2 is a 3.4-kDa peptide.

tography of the ammonium sulfate extract resulted in a peak of A_{280} which contained the majority of lacticin activity. Since this peak eluted towards the end of the chromatogram. lacticin 481 was further separated from high-molecular-weight contaminating proteins. The elution volume of lacticin 481 corresponded to a molecular size of 1.3 kDa. However, this purification step was problematic since only 3.2% of the activity was recovered from the total amount injected into the column. Attempts to improve the recovery of bacteriocin by using Sephadex LH60 (LKB-Pharmacia) with various concentrations of 2-propanol in 0.1% TFA as the mobile phase were not successful. The loss in bacteriocin activity did not appear to be due to hydrophobic interactions of lacticin 481 with the gel matrix. The active extract was purified further by C_{18} reversed-phase HPLC. The elution profile monitored at A_{220} revealed a distinct peak (Fig. 1a. peak 1) which eluted with 34% 2-propanol and correlated with a peak of bactericidal activity. Additional activity also eluted upon washing the column with 80% 2-propanol. The total bactericidal activity recovered in HPLC fractions was significantly higher than that detected in the gel-filtrated extract (Table 1).

To confirm that peak 1 corresponded to lacticin 481. culture supernatants from *L. lactis* subsp. *lactis* T_{H4} (a Bac⁻ Bac' derivative of *L. lactis* subsp. *lactis* T_H that does not produce lacticin 481) was subjected to the above purification

protocol. When loaded on the HPLC C_{18} column, this extract provided the same elution pattern (Fig. 1b) as the producing strain *L. lactis* subsp. *lactis* T_H (Fig. 1a), except that both peaks that correlated with the bacteriocin absorbance peak and bactericidal activity were absent. Therefore. the absorbance peak eluting with bactericidal activity is lacticin 481.

The purity of lacticin 481 was assessed by SDS-PAGE. No stained material was detected on the gel with either Coomassie blue or silver stain. However, electroblotting SDS-polyacrylamide gels to polyvinylidene difluoride membranes concentrated the peptide and allowed visualization of the blotted proteins after being stained with Coomassie blue (Fig. 2). Lacticin 481 from peak 1 (Fig. 1a) appeared as a major band of 1.7 kDa and a minor band of 3.4 kDa (Fig. 2. lane 1). Heating the sample prior to electrophoretic migration did not change the electrophoretic pattern (Fig. 2. lane 3). The two bands were recovered from the membrane blots and tested for inhibitory activity against *L. delbrueckii* subsp. *bulgaricus* 1489. an SDS-resistant indicator strain. Both bands produced zones of inhibition (Fig. 3). although the more abundant 1.7-kDa peptide (B1) generated a larger zone of inhibition than did the 3.4-kDa compound (B2). To purify these two compounds further. an additional purification step was performed on the peak 1 sample by using the C_{18} HPLC column with an ammonium acetate running buffer. Under these conditions. two peaks eluted (Fig. 1c. peaks 2 and 3) that recovered 3 and 97% of the activity. respectively. The two peaks were collected separately and analyzed by SDS-PAGE. A single band of 1.7 kDa (Fig. 2. lane 6) corresponding to peak 3 was detected. whereas peak 2 failed to show a strong signal (lane 5). Amino acid analysis of the two peaks revealed the same composition. indicating that peak 2 was a dimer of peak 1. To confirm the identity of the 1.7-kDa band. fractions derived from the Bac⁻ Bac' *L. lactis* subsp. *lactis* T_{H4}. which were analogous to lacticin 481 eluting fractions. were recovered and analyzed by SDS-PAGE. No 1.7-kDa band or activity was detected (Fig. 2.
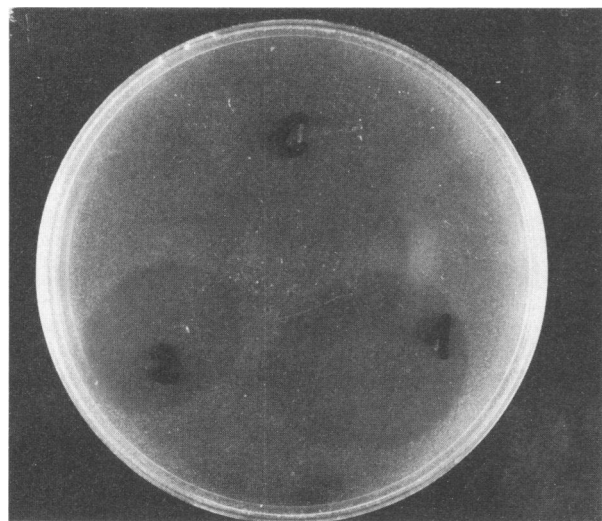
FIG. 3. Antimicrobial activity of band B1 (labelled 1) and B2 (labelled 2) extracted from the SDS-polyacrylamide gel shown in Fig. 2, against *L. delbrueckii* subsp. *bulgaricus* 1489. C, control, which is an extract from a blank blotting membrane.

lane 4), confirming that the 1.7-kDa band purified from *L. lactis* subsp. *lactis* T_{11} was lacticin 481.

Protein sequence analysis. Seven residues (K G G S G V I) were sequenced from the amino terminus of lacticin 481. The remaining peptide was not further degraded by the Edman reaction, and no additional significant signals were detected. No sequence homologous to the lacticin 481 N-terminal sequence was found in the National Biomedical Research Foundation (Washington, D.C.) or Gen Pro (Cambridge, Mass.) data bases.

Amino acid composition. The amino acid composition of purified lacticin 481 is presented in Table 2. Of a total of 18 or 20 amino acids, only 7 are charged, while the remaining are uncharged or nonpolar. Pure lanthionine was used for calibration. When lacticin 481 was analyzed, a clear signal corresponding to 840 pM lanthionine was detected. This corresponded to one or two lanthionine residues per molecule. One tryptophane residue was also detected. No cysteine and no threonine residues were present.

DISCUSSION

Purification of lacticin 481 was achieved with a protocol close to that adopted for lactacin F (25), by using consecutive ammonium sulfate precipitations, gel filtration chromatography, and reversed-phase chromatography. Lacticin 481-containing supernatants were first precipitated at 60% saturation. This step precipitated all lacticin 481 activity and left the maximum amount of extraneous contaminating proteins in the supernatant. Subsequent treatment of the pellet with ammonium sulfate at 80% saturation significantly increased lacticin 481 activity. Dissociation of lacticin 481 appears to have occurred, since the partially purified bacteriocin decreased in molecular size from 5.5 (estimated previously [27]) to 1.3 kDa concurrently with the increase in activity. This phenomenon in which high-molecular-weight complexes were dissociated during purification to their active components with lower molecular weights has been described previously for bacteriocins from *Lactobacillus* spp. (3, 14, 24). However, dissociation of lacticin 481 is not

TABLE 2. Amino acid composition of lacticin 481

Amino acid	No. of residues/molecule[a]		
	Lacticin 481[b]	Nisin[c]	Subtilin[c]
Ala	0.29 (0)	2	1
Asp	1.84 (2)	1	1
Dha[d]	ND[e]	2	2
Dhb[f]	ND	1	1
Glu	2.45 (2)	0	3
Gly	3.14 (3)	3	2
His	1.73 (2)	2	0
Ile	1.03 (1)	3	1
Lan	1.44 (1-2)	1	1
β-CH₃Lan[g]	ND	4	4
Leu	0.30 (0)	2	4
Lys	1 (1)	3	3
Met	0.69 (1)	2	0
Phe	1.67 (2)	0	1
Pro	0.52 (0-1)	1	1
Ser	1.91 (2)	1	0
Trp	0.73 (1)	0	1
Tyr	0.10 (0)	0	0
Val	1.37 (1)	1	1

[a] Mean values from nine runs from three different bacteriocin preparations.
[b] The values in parentheses are the most probable number of the individual amino acids in lacticin 481.
[c] Data per reference 9.
[d] Dha, dehydroalanine.
[e] ND, not determined.
[f] Dhb, dehydrobutyrine.
[g] β-CH₃Lan, β-methyllanthionine.

the only factor affecting variable activity during purification since the size dissociation from 5.5 to 1.3 kDa would only represent a fourfold increase in activity. Fractionation of the ammonium sulfate extract by gel filtration failed to recover greater than 3% of the activity injected. Elution of lacticin 481 activity was not improved even in the presence of organic solvents. Lacticin 481 activity increased again upon reversed-phase chromatography, indicating that the lacticin 481 levels are affected by the chromatographic conditions used. The reason for this behavior is not understood but may be due to conformational modification of lacticin 481.

Chromatography of the lacticin containing extract on a C_{18} reversed-phase column revealed that lacticin 481 is a highly hydrophobic molecule eluting with 34% 2-propanol. This hydrophobicity is consistent with our preliminary results obtained with purification. In anion or cation exchange chromatography, lacticin 481 activity eluted along the entire chromatographic profile (data not presented). In hydrophobic interaction chromatography, lacticin 481 was bound to the matrix in the absence of salt and attempts to remove the activity from the column by running organic solvents were unsuccessful (data not shown). Lacticin 481 can appear as a monomer or as a dimer; with the purification protocol employed, the monomer was favored. These properties are also characteristic of nisin, which appears in mono- or multimeric forms (12, 23). The hydrophobic properties of lacticin 481, as well as other peptide bacteriocins (24, 25), most likely contribute to interprotein interactions that form multimeric complexes and to interactions of these peptides with lipids.

Size estimations of purified lacticin 481 were variable. The molecular size was 1.7 kDa by SDS-PAGE, 1.3 kDa by gel filtration and 2.4 to 2.7 kDa by amino acid composition analysis. This discrepancy probably results from the hydro-

phobicity of lacticin 481 and the presence of thio-ether cross-linkages in lanthionine residues, both of which can lead to an erratic migration in SDS-polyacrylamide gels (25). Similarly, the hydrophobic peptide lactococcin A migrated to a position of 3.4 kDa in SDS-PAGE (33), whereas its calculated molecular size deduced from its amino acid sequence was 5.8 kDa (11).

A few other lanthionine-containing peptide antibiotics have been purified by using a variety of techniques. Nisin has been purified by organic solvent precipitations (5), countercurrent distribution (4), or anion exchange chromatography plus pH gradient chromatography (2). Epidermin was purified by adsorption on amberlite XAD-8, gel filtration, and countercurrent distribution (1), while gallidermin and pep5 were purified by reversed-phase HPLC (16, 17). These methods provided purification yields of 10 to 50%, but none of these studies reported an increase in total biological activity similar to that of lacticin 481 treated with ammonium sulfate.

Amino acid composition analysis revealed that lacticin 481 contained a majority of nonpolar amino acids and some lanthionine residues. No striking similarities in amino acid composition between lacticin 481 and other lanthionine-containing peptides, such as nisin, subtilin (Table 2), gallidermin (17), epidermin (1), or pep5 (16), were observed. The α- and β-unsaturated amino acids (dehydroalanine and dehydrobutyrine), which are present in nisin and subtilin, could not be identified in acid hydrolysates of lacticin 481, since they are subject to degradation during acid hydrolysis (9). However, the absence of threonine in lacticin 481 could indicate that this residue has been dehydrated to dehydrobutyrine. No cysteine was found in lacticin 481, and this could be the result of lanthionine or β-methyllanthionine formation. The presence of unusual amino acids in lacticin 481 explains some of the observations made during this study. Lanthionine is known to introduce a high level of hydrophobicity (16). Also, the α- and β-unsaturated amino acids are very reactive (23) and could explain the formation of dimers. In automated Edman degradation reactions used for amino acid sequencing, lanthionine residues should provide blank cycles (17), but α- or β-dehydrated amino acids may terminate the reaction (17). No blank cycle was observed during sequencing of the first seven N-terminal residues, indicating that no lanthionine is present in this portion of lacticin 481. This contrasts with most lantibiotics, i.e., nisin (9), subtilin (9), epidermin (1), and gallidermin (17), which contain a lanthionine residue at position 3. However, the termination of Edman degradation at the eighth residue could be due to the presence of a dehydrated residue in this position. The N-terminal amino acid sequence of lacticin 481 did not reveal homology with any known sequence, suggesting that lacticin 481 is a new lantibiotic.

Lanthionine-containing antimicrobial peptides are a growing family of compounds which have received the name lantibiotics (31). These peptides are unique in that they are ribosomally synthesized as prepeptides and undergo post-translational processing of a number of amino acids (serine, threonine, and cysteine) into dehydro residues and thio-ether crossbridges. Lantibiotics are produced by a diverse number of gram-positive bacteria, including both GRAS (generally recognized as safe) organisms, such as *L. lactis* subsp. *lactis* (nisin), and pathogenic strains, such as *Staphylococcus epidermidis* (epidermin and pep5) (1, 16). In spite of peptide similarities which indicate that these peptides have evolved from a common ancestor (15), different and variable spectra of bactericidal activities are observed.

These features may suggest that different structural analogs with varied properties could be available naturally or constructed by site-directed mutagenesis. Compared with nisin, lacticin 481 exhibits a narrower spectrum of activity, a smaller size, and a higher stability at neutral pH. In the short term, lacticin 481 could be an effective food-grade lantibiotic for inhibition of spoilage lactic acid bacteria and clostridia in some food products (alcoholic beverages, meat products, and vegetables packed under controlled atmosphere). In the longer term, comparison of lacticin 481 with nisin and with other lantibiotics may provide a consensus of structure-function relationships that defines particular peptide sequences or structures important to inhibitory activity. Work is in progress to elucidate the molecular structure of lacticin 481.

REFERENCES

1. **Allgaier, H., G. Jung, R. G. Werner, U. Schneider, and H. Zähner.** 1986. Epidermin: sequencing of a heterodet tetracyclic 21-peptide amide antibiotic. Eur. J. Biochem. **160:**9–22.
2. **Bailey, F. J., and A. Hurst.** 1971. Preparation of a highly active form of nisin from *Streptococcus lactis*. Can. J. Microbiol. **17:**61–67.
3. **Barefoot, S. F., and T. R. Klaenhammer.** 1984. Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. Antimicrob. Agents Chemother. **26:**328–334.
4. **Berridge, N. J., G. F. Newton, and E. P. Abraham.** 1952. Purification and nature of the antibiotic nisin. Biochemistry **52:**529–534.
5. **Cheeseman, G. C., and N. J. Berridge.** 1957. An improved method of preparing nisin. Biochem. J. **65:**603–608.
6. **Davey, G. P., and B. C. Richardson.** 1981. Purification and some properties of diplococcin from *Streptococcus cremoris* 346. Appl. Environ. Microbiol. **41:**84–89.
7. **Edman, P., and G. Begg.** 1967. A protein sequenator. Eur. J. Biochem. **1:**80–81.
8. **Geis, A., J. Singh, and M. Teuber.** 1983. Potential of lactic streptococci to produce bacteriocin. Appl. Environ. Microbiol. **45:**205–211.
9. **Gross, E.** 1977. Chemistry and biology of amino acids in food proteins. Lysinoalanine. p. 37–51. *In* R. E. Feeney and J. R. Whitaker (ed.), Food proteins: improvement through chemical and enzymatic modification. American Chemical Society, Washington, D.C.
10. **Henschen, A.** 1987. Analysis of cyst(e)ine residues, disulfide bridges, and sulfhydryl groups in proteins. p. 244–255. *In* B. Wittman-Liebold, J. Salnikow, and V. A. Erdmann (ed.), Advanced methods in protein microsequence analysis. Springer-Verlag, Berlin.
11. **Holo, H., O. Nilssen, and I. F. Nes.** 1991. Lactococcin A, a new bacteriocin from *Lactococcus lactis* subsp. *cremoris*: isolation and characterization of the protein and its gene. J. Bacteriol. **173:**3879–3887.
12. **Hurst, A.** 1981. Nisin. p. 85–123. *In* D. Perlman and A. I. Laskin (ed.), Advances in applied microbiology, vol. 27. Academic Press, New York.
13. **Ingram, L.** 1970. A ribosomal mechanism for synthesis of peptides related to nisin. Biochim. Biophys. Acta **224:**263–265.
14. **Joerger, M., and T. R. Klaenhammer.** 1986. Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helve-*

ticus 481. J. Bacteriol. **167**:439–446.

15. **Kaletta, C., and K.-D. Entian.** 1989. Nisin, a peptide antibiotic: cloning and sequencing of the *nisA* gene and posttranslational processing of its peptide product. J. Bacteriol. **171**:1597–1601.

16. **Kaletta, C., K.-D. Entian, R. Kellner, G. Jung, M. Reis, and H.-G. Sahl.** 1989. Pep5, a new lantibiotic: structural gene isolation and prepeptide sequence. Arch. Microbiol. **152**:16–19.

17. **Kellner, R., G. Jung, T. Hörner, H. Zähner, N. Schnell, K.-D. Entian, and F. Götz.** 1988. Gallidermin: a new lanthionine-containing polypeptide antibiotic. Eur. J. Biochem. **177**:53–59.

18. **Klaenhammer, T. R.** 1988. Bacteriocins of lactic acid bacteria. Biochimie **70**:337–349.

19. **Kozak, W., J. Bardowski, and W. Dobrzanski.** 1978. Lactostrepcins-acid bacteriocins produced by lactic streptococci. J. Dairy Res. **45**:247–257.

20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.

21. **Lindgren, S. E., and W. J. Dobrogosz.** 1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. Rev. **87**:149–164.

22. **Liu, T. Y., and R. A. Boykins.** 1989. Hydrolysis of proteins and peptides in a hermetically sealed microcapillary tube: high recovery of labile amino acids. Anal. Biochem. **182**:383–387.

23. **Liu, W., and J. N. Hansen.** 1990. Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. Appl. Environ. Microbiol. **56**:2551–2558.

24. **Mørtvedt, C. I., J. Nissen-Meyer, K. Sletten, and I. F. Nes.** 1991. Purification and amino acid sequence of lactocin S, a bacteriocin produced by *Lactobacillus sake* L45. Appl. Environ. Microbiol. **57**:1829–1834.

25. **Muriana, P. M., and T. R. Klaenhammer.** 1991. Purification and partial characterization of lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088. Appl. Environ. Microbiol. **57**:114–121.

26. **Piard, J.-C., F. Delorme, G. Giraffa, J. Commissaire, and M. Desmazeaud.** 1990. Evidence for a bacteriocin produced by *Lactococcus lactis* CNRZ 481. Neth. Milk Dairy J. **44**:143–158.

27. **Piard, J.-C., and M. Desmazeaud.** Inhibiting factor produced by lactic acid bacteria. 1. Oxygen metabolites and catabolism end-products. Lait, in press.

28. **Piard, J.-C., and M. Desmazeaud.** Inhibiting factor produced by lactic acid bacteria. 2. Antibacterial substances and bacteriocins. Lait, in press.

29. **Pluskal, M. G., M. B. Przekop, M. R. Kavonian, C. Vecoli, and D. A. Hicks.** 1986. Immobilon PVDF transfer membrane: a new membrane substrate for Western blotting of proteins. Biotechniques **4**:272–283.

30. **Rammelsberg, M., E. Müller, and F. Radler.** 1990. Caseicin 80: purification and characterization of a new bacteriocin from *Lactobacillus casei*. Arch. Microbiol. **154**:249–252.

31. **Schnell, N., K.-D. Entian, U. Schneider, H. Götz, H. Zähner, R. Kellner, and G. Jung.** 1988. Prepeptide sequence of epidermin, a ribosomally synthetized antibiotic with four sulphide-rings. Nature (London) **333**:276–278.

32. **Sober, H. A. (ed.).** 1968. Handbook of biochemistry, selected data for molecular biology. CRC Co., Cleveland.

33. **van Belkum, M. J., B. J. Hayema, R. E. Jeeninga, J. Kok, and G. Venema.** 1991. Organization and nucleotide sequences of two lactococcal bacteriocin operons. Appl. Environ. Microbiol. **57**:492–498.